## REMARKS/ARGUMENTS

Favorable consideration of this application, as presently amended and in light of the following discussion, is respectfully requested.

Claims 1, 5-7, and 9-18 are presently pending in this application, Claims 2-4 and 8 having been canceled, and Claims 1, 5-7, 9, and 12 having been amended by the present amendment.

In the outstanding Office Action, Claims 1-2, 5-10, and 12-17 were rejected under 35 U.S.C. §102(b) as being anticipated by EP 0 282 009 (hereinafter "EP '009"); and Claims 3-4, 11, and 18 were rejected under 35 U.S.C. §103(a) as being unpatentable over EP '009.

Claim 1 has been amended to include the subject matter recited in Claims 2-4 as originally filed. Claims 5-7 and 9 which depend from Claim 1 have been amended accordingly. Claim 12 has been amended similar to Claim 1. Applicants therefore submit that no new matter has been introduced.

Briefly, the present invention as recited in Claim 1 is directed to a method of monitoring for the presence of hydrophobic liquid at a site. At the site, a sensor assembly including a polyvinylidene fluoride membrane is located. The polyvinylidene fluoride membrane is adapted to take up hydrophobic liquid from the site. The sensor assembly further includes: radiation input means connected to a radiation source and arranged to irradiate the membrane; and radiation output means connected to a radiation detector and/or analyser arranged to detect and/or analyse radiation which results from the irradiation of the membrane by the radiation input means. In this method, the radiation input means is caused to irradiate the membrane, and the detector/analyser is employed to receive radiation via the radiation output means. The arrangement is such that the nature and/or amount of radiation received by the detector/analyser is affected by the presence of liquid at the site. By

providing a sensor assembly including a polyvinylidene fluoride membrane, hydrophobic liquid is more efficiently detected while water is repelled from the sensing surface.[1]

EP '009 discloses a fibre-optic detector for oils and solvents. Nevertheless, EP '009 does not teach "locating at said site a sensor assembly which comprises *a polyvinylidene fluoride membrane* which is adapted to take up hydrophobic liquid from the site" as recited in amended Claim 1 (emphasis added in Italic). On the other hand, EP '009 discloses a polytetrafluoroethylene (hereinafter "PTFE") film for use in the sensor.[2] EP '009 provides no teaching pointing towards any other fluorocarbons, and certainly no suggestion of polyvinylidene fluoride (hereinafter "PVF").

Furthermore, Applicants respectfully submit experimental data supporting superiority of a PVF membrane over a PTFE membrane for taking up hydrophobic liquid from the site.[3] Extensive experiments were performed by using PVF, PTFE and other membrane materials, and their results clearly show advantageous properties of the PVF membrane. For example, unlike the PTFE membrane, the PVF membrane demonstrated full reversibility of the contaminated membrane signal showing complete and rapid recovery from multiple contamination by evaporation of the contaminants in air. Moreover, the PVF membrane was physically the most flexible and durable membrane when compared to other membranes, thus more suitable for filed-based use with the sensor.[4]

Therefore, the method recited in Claim 1 is believed to be patentably distinguishable from EP '009. Hence, Claim 1 is believed to be allowable.

Likewise, Claim 12 includes subject matter substantially similar to what is recited in Claim 1 to the extent discussed above. Thus, Claim 12 is also believed to be allowable.

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[1] See Specification, page 11, lines 26-28.
[2] See EP '009, column 4, lines 37-39.
[3] See Attachment A, "Membrane evaluation for incorporation with the oil & fuel leak sensor."
[4] See id., Experiments 2 and 3.

Furthermore, since Claims 5-7, 9-11, and 13-18 ultimately depend from either Claim 1 or 12, substantially the same arguments set forth above also apply to these dependent claims. Hence, Claims 5-7, 9-11, and 13-18 are believed to be allowable as well.

In view of the amendments and discussions presented above, Applicants respectfully submit that the present application is in condition for allowance, and an early action favorable to that effect is earnestly solicited.

Respectfully submitted,

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Attachment A



# Membrane evaluation for incorporation with the oil & fuel leak sensor

## Summary

Fluorotrans demonstrated a number of properties that render it a superior material compared to other membranes for the desired application:

1. A wide selection of membranes with different optical and filtration properties and potential hydrocarbon affinities were evaluated for potential use with the oil/fuel sensor (Appendix 1). Each was tested in turn with 3 different fuel types: central heating oil, standard diesel and unleaded petrol (Experiment 1)

2. The opacity of uncontaminated Fluorotrans membrane enabled a consistently low transmittance background [baseline] signal compared to other membranes tested (Experiment 1)

3. Fluorotrans membrane demonstrated maximum translucency and correspondingly maximum signal responses to the three different fuels, thus enabling maximum sensor sensitivity (Experiment 1)

4. Only Fluorotrans membrane demonstrated full reversibility of the contaminated membrane signal (with unleaded petrol & diesel) showing complete and rapid recovery from multiple contamination by evaporation of the contaminants in air.

5. Fluorotrans membrane exhibited the property of reverting back to initial baseline levels after multiple contamination with all fuels tested and did so more rapidly than any other membrane evaluated during this study (Experiment 2)

6. Contamination of the Fluorotrans membrane was also removed (or reversed) by dissolution when the sensor was placed in water. However, the recovery time was significantly extended in water and influenced by fuel type (Experiment 3)

7. Fluorotrans was physically the most flexible and durable membrane when compared to other membranes, thus more suitable for field-based use with the sensor. Other membranes showed degradation and weakening from fuel contamination, indicated by the increased baseline values and low fuel loading capacity (Experiments 2 & 3).

8. Additionally, it was possible to distinguish between fuel types (for example diesel and unleaded petrol) by monitoring the signal kinetics from the contaminated Fluorotrans membrane in both air and water matrices.

## Experiment 1. To demonstrate improved baseline signal for FT and high signal response to fuel contamination compared to other commercially available membranes

### Aims & Methods

In order to achieve maximum sensor sensitivity, a low baseline (background) signal is required, which corresponds to the high opacity (low light transmittance) of the uncontaminated membrane. Similarly, when in contact with a small amount of fuel contamination (which causes membrane translucency), a high signal response (≥ 2.4V) is required in order to achieve the maximal signal difference between uncontaminated and contaminated membrane. Whilst the sensor capacity is 2.5V, in practical applications, the maximum achievable signal is 2.4V. This is attributed to limitations of the data acquisition unit employed, as current will be lost during the transmission of data, hence a small degree of signal loss.

For the signal comparison experiments, the data acquisition programme was set to sample the sensor signals every second. A sensor was held ~~with~~in a clamp within a fume hood and used repeatedly for each test. Between each of the membrane and/or fuel evaluations, the sensor head was cleaned thoroughly with methanol. Membranes were held flush to the sensor face using an O-ring. Initial baseline values were established for ~ 10s for each membrane tested to determine the degree of initial opacity. After this time, 16μl of each fuel was manually pipetted onto the membrane surface and the responses monitored for ~ 50 s.

### Results & Discussion

<u>**Central Heating Oil**</u>

Figure 1 shows the membrane responses to central heating oil. Maximum signal (≥2.4V) was only obtained with Fluorotrans and PTFE tape. However, whilst Fluorotrans had a minimum baseline signal ≤ 0.1V, PTFE tape exhibited a high baseline signal of ~1.2V, thus showing a degree of light transmission prior to contamination. Good responses (> 2V) were also exhibited by Polypropylene and GHP450 membrane, although these were more variable. The Versapore membrane, which was recommended for the specific task by the membrane suppliers (Pall Europe Ltd., Portsmouth, UK) showed no response to contamination by central heating oil. The baseline signal remained constant and this was kept for further testing as a potential control membrane.

The two Biodyne membranes both produced high baseline signals, coupled with poor responses to contamination, and so these were not evaluated further. The Mycelx membrane was designed for use in fuel spill recoveries. Although this showed a poor response when used as a detection membrane for central heating oil, it was tested further using the other fuels.

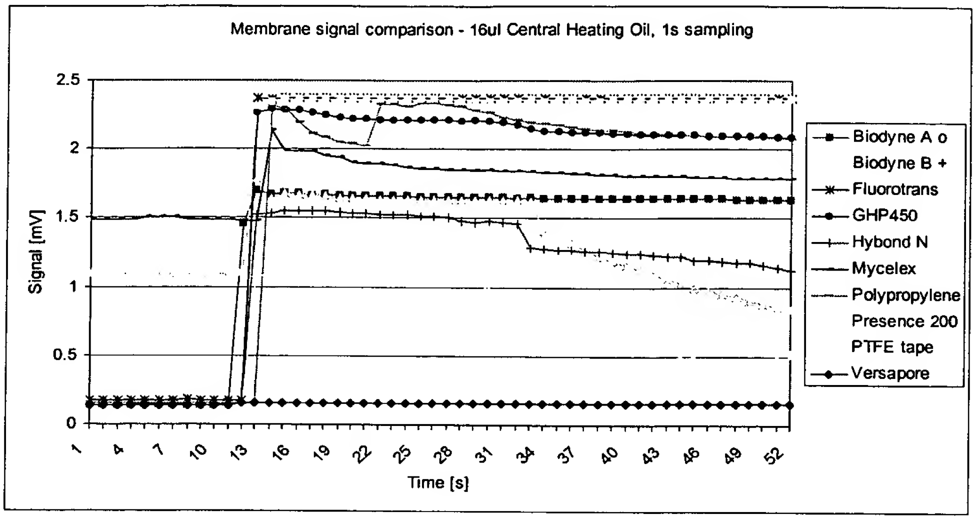


**Figure 1. Membrane response to central heating oil**
Maximum signal was only obtained by Fluorotrans and PTFE tape. Good responses (> 2V) were also exhibited by Polypropylene and GHP450 membrane. Versapore showed no response to contamination, indicated by the constant baseline signal.

## Diesel

Figure 2 shows membrane responses to 16µl diesel contamination. Overall, only the Fluorotrans membrane demonstrated a low background and high signal response to diesel fuel and this was comparable to the response of this membrane to central heating oil.

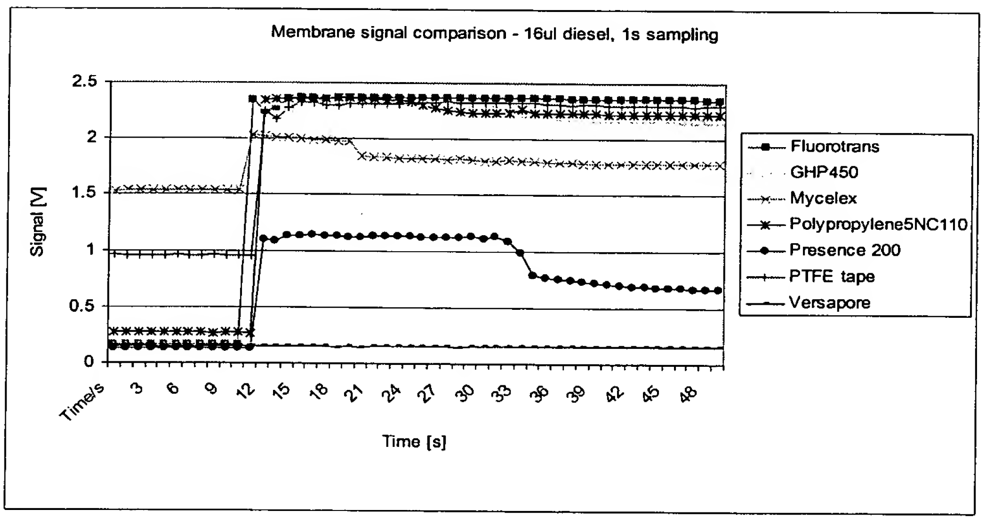


**Figure 2. Membrane response to diesel contamination**
Fluorotrans demonstrated the highest response to diesel contamination. GHP450, polypropylene and PTFE also had good responses (≥ 2.3V) although more signal fluctuation was observed.

## Unleaded Petrol

Unleaded petrol was the last fuel evaluated (Figure 3). Again, Fluorotrans produced the highest response to contamination. In contrast to the heavier hydrocarbon fuels previously evaluated, all membranes, with the exception of Mycelx and Versapore exhibited evidence of signal reversibility. This may be due to the lighter hydrocarbon fractions present in petroleum fuels and the greater percentage of volatile compounds. The Mycelx again showed a very high baseline and a poor response. Versapore membrane did not respond to any fuels.

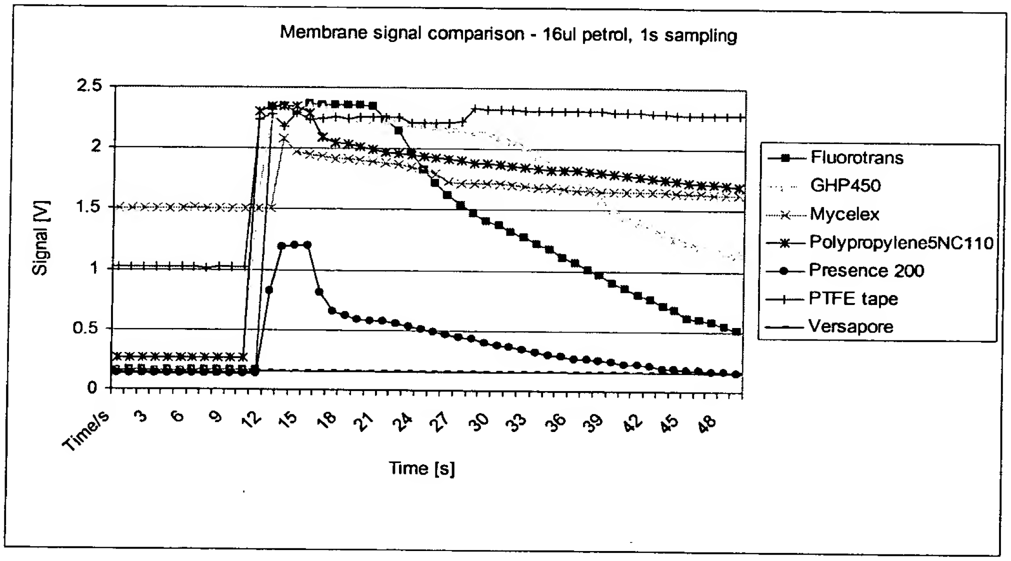


**Figure 3. Membrane response to unleaded petrol contamination**
Fluorotrans demonstrated the highest response to petrol contamination. GHP450, polypropylene and PTFE also had good responses (≥ 2.3V) although more signal fluctuation was observed.

## Experiment 2. To demonstrate membrane response to repeated contamination and reversibility by evaporation in air

### Aims & Methods

The 3 membranes with the highest signal responses to all three fuels were: Fluorotrans, polypropylene and PTFE tape. Tests for reversibility of the membrane were thus conducted only on these three membranes.

As with the previous experiments, the sensor used for all membrane evaluations was held in place using a clamp to ensure there were no environmental fluctuations which may have interfered with the signal responses, for example light fluctuations. A constant temperature of 20° C was maintained.

A baseline signal for each membrane was initially established for ~10s. Following this, 8μl of fuel was placed onto the membrane surface and the response monitored for 60s. A further 8μl of fuel was then added, regardless of current signal. This was

repeated for a total of 3 fuel contaminations to show membrane loading capacity. After the last fuel addition, signal monitoring was continued to determine whether the signal returned (reversed to the original baseline level. Further 8μl aliquots of fuel were subsequently added and the sensor monitoring continued.

## Results & Discussion

Fluorotrans showed renewed response to 3 consecutive contaminations of 8μl petrol at 60s intervals (Figure 4). The signal was then monitored until it returned to the initial baseline value. PTFE showed no response to further contamination after 16μl petrol loading onto the membrane surface. Fluorotrans membrane quickly returned to the initial baseline value (+/- 1%), whilst the other two membranes retained a degree of fuel residue and so increased their baseline values (in the time for which they were monitored). Fluorotrans demonstrated the quickest reversibility (evaporation/recovery time) when contaminated with unleaded petrol and left to evaporate in air (~ 3.2 minutes) with the signal returning to the baseline value of 0.3V after each subsequent contamination. The signal from the Polypropylene membrane was also reversible but with a much slower decay time of >11 minutes. The PTFE signal took 34 minutes to return to baseline after only 16μl petrol contamination.

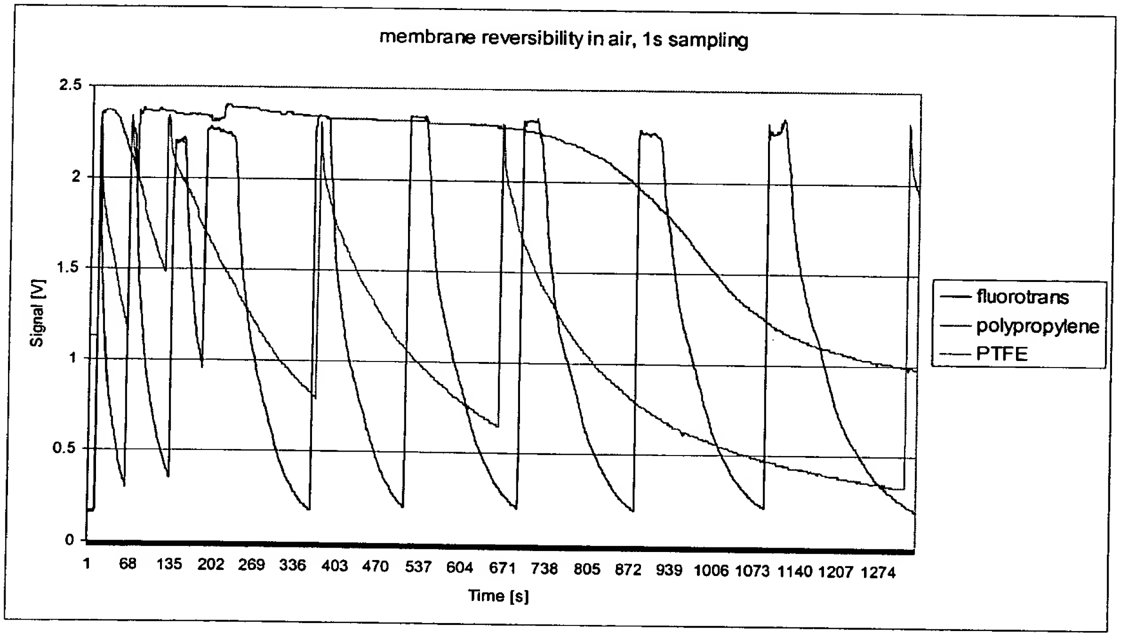


**Figure 4. Membrane signal reversibility, petrol contamination in air**
Fluorotrans demonstrated the quickest reversibility (evaporation/recovery time) when contaminated with unleaded petrol and left to evaporate in air with the signal reaching the baseline value of 0.3V on each subsequent contamination and recovery.

The tests were repeated using standard grade diesel fuel (Figure 5).

Whilst all three membranes showed a rapid and high response to 8μl diesel contamination, the retention of the fuel by the membrane was greatly increased with the heavier diesel fuel, as compared to petrol. Only the Fluorotrans membrane showed reduction in signal over an extended 35 minute monitoring period. Monitoring was continued with 5 minute sampling in order to assess reversibility (Figure 6).

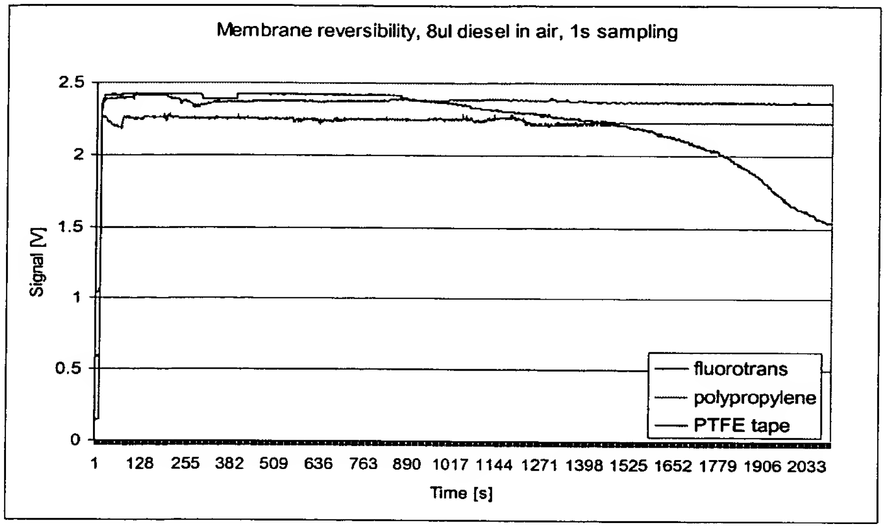


**Figure 5. Membrane signal reversibility, diesel contamination in air**
Only the Fluorotrans membrane showed reduction in signal over an extended 35 minute monitoring period. PTFE or polypropylene showed no reversibility over this time period.

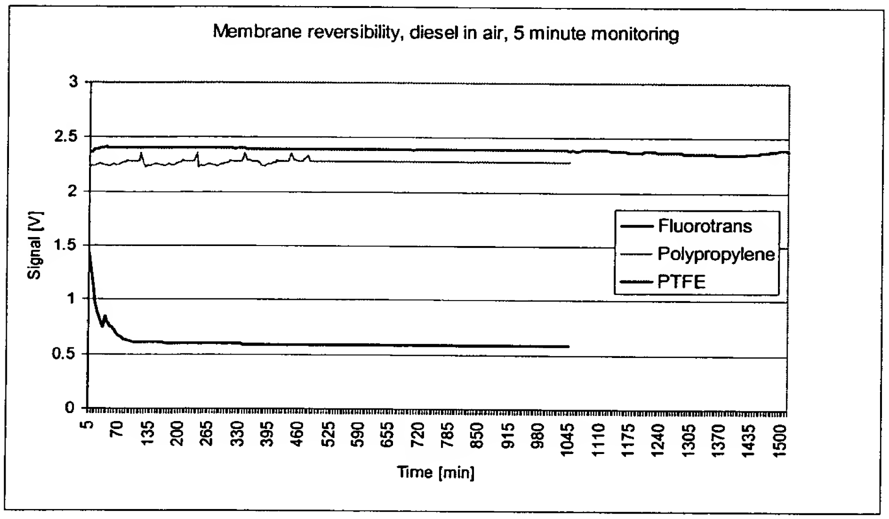


**Figure 6. Extended monitoring of diesel contamination in air**
Further monitoring the signal response every 5 minutes, Fluorotrans continued to show reversibility in air, with the signal falling back to an initial background level of 0.59V. After some small fluctuation, the polypropylene and PTFE membranes stabilised and showed no diesel signal reduction even after 25 hours.

Thus, it was concluded that it was possible to demonstrate full reversibility of the signal from the Fluorotrans membrane, with complete recovery from repeated contamination when the fuel was allowed to evaporate in air.

## Experiment 3. To demonstrate membrane response to repeat contamination and reversibility by dissolution in water

### Aims & Methods

Removal of contaminants from the membrane surface *in situ* (within a water matrix) was also desirable. For evaluation of the membrane signal reversibility in water, experiments with petrol and diesel contamination were repeated using Fluorotrans, polypropylene and PTFE membrane. As with the previous Experiments 1 & 2, the sensor used for all membrane evaluations was held in place using a clamp a constant room temperature of 20° C was maintained.

A baseline signal for each membrane was initially established for ~10s. Then, 8µl of fuel was pipetted onto the membrane surface. In contrast to Experiment 2, the sensor head and attached membrane was then immediately immersed ~~into~~ a beaker containing 400ml water. The water was moderately agitated using a magnetic stirrer. The sensor response was then monitored for 60s. A further 8µl of fuel was then added, regardless of current signal. (for this and subsequent contaminations, the sensor was removed from the water and then immediately placed back into the solution). Unless otherwise stated, this was repeated for a total of 3 fuel contaminations to show the membrane loading capacity. After the last fuel addition, monitoring was continued to see if the membrane signal reversed to the original baseline level (Figure 7).

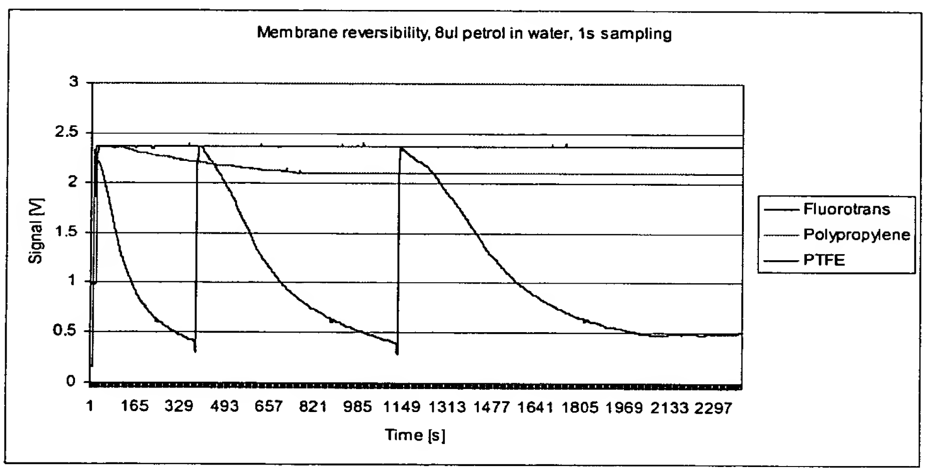


**Figure 7. Membrane signal reversibility, petrol contamination in water**

Only the Fluorotrans membrane demonstrated repeated recovery from petrol contamination within a water matrix. This took 12 minutes.

The Fluorotrans membrane recovered to a slightly higher but consistent baseline value of 0.5V after ~ 12 minutes (the original baseline was 0.17V).This was attributed to a visible residue on the membrane. Maximum response consistently reached 2.37V. PTFE showed no loss in signal after contamination. Polypropylene showed some loss of signal and was therefore monitored further (Figure 8).

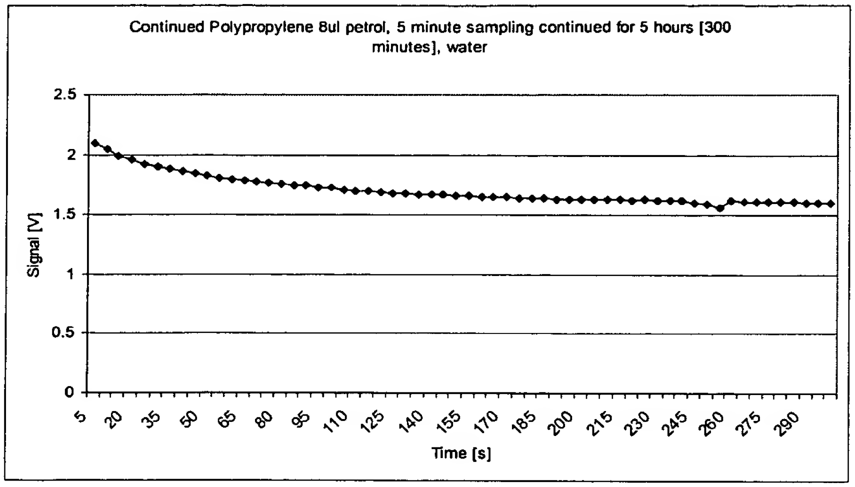


**Figure 8. Extended monitoring of polypropylene signal reversibility in water**
After continuing with 5 minute sampling for 5 hours, the signal from polypropylene fell & stabilised at 1.61V. No further signal reduction was observed. A significant proportion of fuel was retained on the membrane, because the initial baseline value for polypropylene was <0.2V (Figure 7).

It was concluded that the Fluorotrans membrane showed superior reversibility after petrol contamination in both air & water matrices. The response to diesel contamination was then examined (Figure 9).

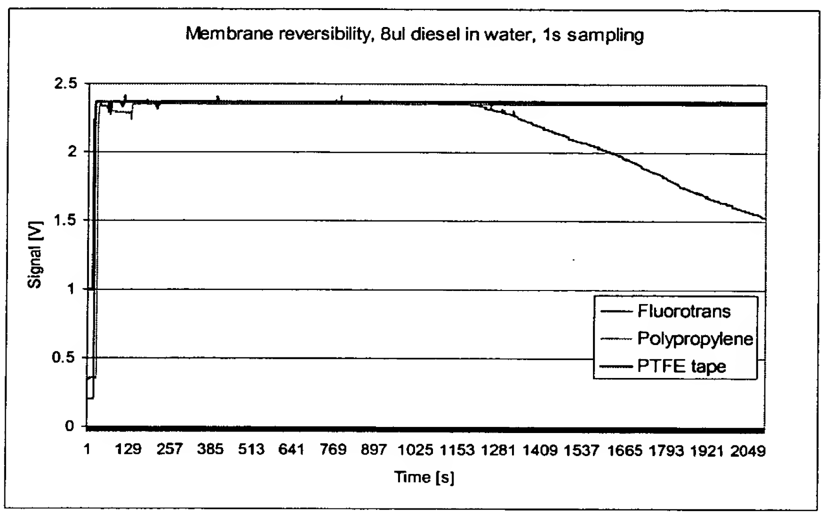


**Figure 9. Membrane signal reversibility after diesel contamination in water**
Fluorotrans membrane was the only membrane to show a reduction in signal after time when immediately placed into water after 8µl diesel contamination followed by immersion in water.

Fluorotrans was the only membrane to show signal reversibility in water after 8µl diesel contamination. The other membranes failed to show any change in signal after 30 minutes. Monitoring was again extended with 5 minute sampling over a 25 hour period (Figure 10).

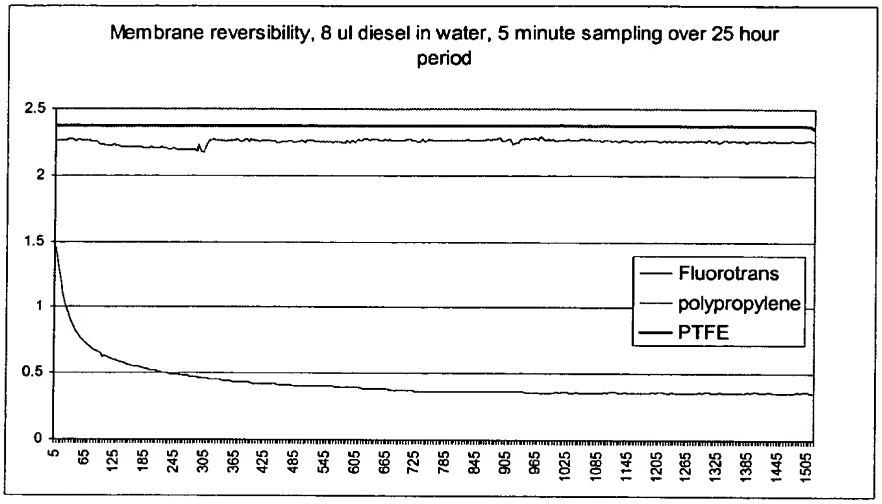


**Figure 10. Extended monitoring of membrane signal reversibility in water after contamination with diesel**

As with petrol contamination, the signal from the Fluorotrans membrane was the only membrane to reverse back to the initial baseline value of 0.4V. Over 25 hours in stirred water, the other two membranes gave no indication of signal reversibility.

## Experiment 4. Demonstrating the ability of Fluorotrans to differentiate fuel types

### Aims & Methods

From the results of Experiments 2 & 3, it was determined that the light transmittance signal obtained when the sensor membrane became translucent (upon hydrocarbon contamination) was fully and rapidly reversible only in the case of the Fluorotrans membrane. This was observed in both air and water matrices. During these experiments, the potential ability of the Fluorotrans membrane to differentiate between two primary fuel types (unleaded petrol and diesel) was investigated further.

### Results

The hypothesis was that the rate of migration of the contaminant from the membrane could be achieved through evaluating the signal kinetics in response to contamination by each fuel type and in each matrix (air and water). The way in which Fluorotrans may be used to distinguish fuel types is that the rate of signal reversal (or rate of signal decay) is a defining characteristic of the contaminating hydrocarbon mixture. Therefore, this can then be employed as a method of distinguishing one hydrocarbon mixture from another. Taking diesel and petrol as examples, the underlying hypothesis is that the retention time of the diesel on the membrane (in air or water) would be much higher than that of petrol (because of its different chemical properties such as solubility and volatility). Consequently, the response signal would remain high.

Conversely, petrol contamination would rapidly evaporate (in air) or be removed from the membrane surface by dissolution (in water) and so the response signal would reverse more rapidly. This can be particularly attributed to the more highly volatile and soluble components present in petrol, (for example, fuel oxygenates), which are not present in the heavier diesel fuel. Eventually, the sensor signal reverts back to the original baseline (background) value ($\leq$0.5V). This difference in the kinetics of the reversibility of the Fluorotrans response to petrol and diesel was demonstrated clearly in Experiments 2 & 3.

## Conclusions

Fluorotrans demonstrated the maximum translucency and correspondingly maximum signal responses to all three fuels evaluated. In addition, the opaque membrane displayed a consistently low background [baseline] signal, thus enabling maximum sensor sensitivity.

The Fluorotrans membrane also exhibited the important property of signal reversibility back to baseline levels after repeated contaminations with all the fuels tested and did this more rapidly than any other membrane evaluated during this study. Reversibility was demonstrated in both air and water.

Fluorotrans was also the most flexible and durable membrane when compared to the other membranes and was thus more suitable for field-based use with the sensor. Other membranes showed degradation and weakening after fuel contamination, indicated by the increased baseline values and low fuel loading capacity (Experiments 2 & 3).

It was also possible to distinguish between different fuel types (diesel and unleaded petrol are examples) by monitoring the signal kinetics from the contaminated membrane. Petrol and diesel will migrate from only the Fluorotrans membrane in both air and water matrices. Thus, the rate of migration can distinguish between the two fuels, with retention time of the diesel much higher than that of petrol. This is attributed to the more volatile and soluble components present in petrol, (such as fuel oxygenates), which are not present in the heavier diesel fuel. The rate of contaminating hydrocarbon removal from the membrane within a saturated soil (when the sensors are deployed in the field) would probably depend on the soil type and its properties, (particularly water content and hydrolytic conductivity). It is envisaged that evaporation of the more volatile petroleum compounds from the membrane would be more efficient in air or dry soil, than by removal of contaminant by dissolution in water.

# Appendix 1 – Membrane properties

| | |
|---|---|
| Biodyne Nylon 6,6 | Solid phase membrane assays. Solvent compatible. Intrinsic hydrophilicity.<br>A – amino & carboxyl groups<br>B – ammonium groups. Positively charged for ionic binding |
| Emflon PTFE membrane/tape | Repels low-surface tension liquids, wide chemical compatibility |
| Fluorotrans PVDF | Hydrophobic polyvinylidine fluoride, solid phase support binds via hydrophobic interactions. Enables detection with common staining agents, high tensile strength and chemical resistance. |
| GHP450 (GH polypro) | Universal treated polypropylene membrane, fluorescent detection of analytes |
| Mycelx | MYCELX is a patented polymeric surfactant technology. Bonds to hydrocarbons etc. to make them hydrophobic and viscoelastic thus removing them permanently from the water stream. www.mycelx.com |
| Presense | Hydrophilic polyethersulfone, tensile strength, enhanced reflectance |
| Versapor R membrane | Treated acrylic co-polymer nylon support membrane, Fluororepel treated for superior hydrophobicity, wide chemical compatibility |

- All membranes (except Mycelx) were obtained from Pall Europe Ltd., Portsmouth, UK. www.pal.com